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Clinical and Molecular differences of hypertensive disorders during pregnancy

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Abstract

Background: Hypertensive disorders of pregnancy (HDP) comprise a spectrum of four subtypes: chronic hypertension (cHTN), gestational hypertension (gHTN), preeclampsia (PE), and superimposed preeclampsia (siPE). Although often characterized as a spectrum of disease severity, there have been limited comparative studies of detailed clinical and molecular characteristics of these disorders. We hereby evaluate HDP subtypes using clinical, placental histopathological, and molecular data to compare similarities and differences between HDP subtypes.

Methods: We utilized data from an over 10-year-long pregnancy cohort with detailed clinical and placental pathology, as well as placental tissue RNA-sequencing, to compare findings between HDP subtypes using a nested case-control design. Clinical diagnosis was based on current ACOG criteria, and placental gross and histologic examination was based on the Amsterdam consensus statement.

Results: Clinical data analysis showed cHTN and gHTN to be more likely to have normal placental pathology, while PE and siPE were more enriched in maternal vascular malperfusion

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Author contributions

MH and MBJ designed the research; AE, TNL, SLB, SA, MM, and VS recruited patients and collected clinical information; MBJ collected and managed database; LLS, RW reviewed the data within the obstetrics registry and adjudication of hypertensive disease of pregnancy; OA reviewed placental pathology data and coding of data into the obstetrics registry; JC, AH, and AE performed RNA extraction and preprocessing/mapping of the RNA-seq data, MH and MBJ performed clinical data analysis of clinical and pathologic data; MH, MBJ, and RM performed computational data analysis; MH, MBJ, and RM wrote the manuscript text, and all the authors reviewed the final version of the manuscript.

Disclosures

None

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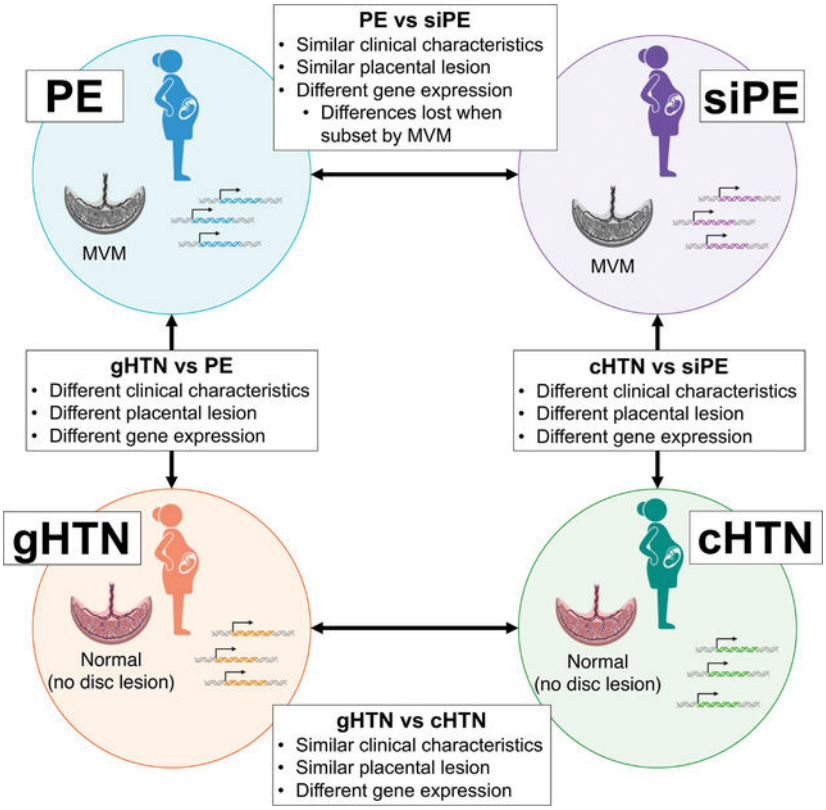
(MVM). RNA-seq showed distinct gene expression signatures and pathway activation across HDP subgroups. We could not identify any molecular evidence that preeclampsia (PE or siPE) was an advanced stage of hypertensive disorder (gHTN or cHTN), but rather identified distinct gene expression profiles between these entities, suggesting preeclampsia (PE or siPE) and hypertension (gHTN or cHTN) are distinct pathophysiological conditions. Finally, we found that, in the presence of MVM, siPE and PE share significant gene expression profiles and pathway activation.

Conclusion: Our findings suggest that MVM specifically differentiates pregnancies that progress to PE and siPE. MVM is thought to initiate in early gestation, indicating the cascade to PE/siPE may be differentiated from gHTN/cHTN early in pregnancy. Incorporating placental histopathological evaluation is an essential future avenue in probing the etiology of HDP.

Graphical Abstract



Keywords

Hypertensive disorder of pregnancy; chronic hypertension; gestational hypertension; preeclampsia; superimposed preeclampsia; maternal vascular malperfusion

Introduction

Hypertensive disorders of pregnancy (HDP) have been recategorized over the years due to new understanding of the disease, leading to improved clinical diagnosis and focused research on its pathophysiology. The most recent classification of HDP includes four

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major hypertensive disorders that occur in pregnant women: chronic hypertension (cHTN), gestational hypertension (gHTN), preeclampsia (PE), and superimposed preeclampsia (siPE). The differential diagnosis within HDP is based on the timing of hypertension onset (before or after 20 weeks of gestation) and the severity of the disease (presence or absence of preeclampsia symptoms, such as proteinuria or other end-organ dysfunctions)[1]. HDP prevalence has been rising over the years, likely due to increased maternal age, obesity, and the higher prevalence of underlying medical conditions, leading to increased maternal and fetal morbidity and mortality[2]. Thus, there is an increasing need for improved early detection markers and fundamental interventions to improve long-term health of both mothers and infants, as well as to reduce the burden on the health care system.

There have been multiple epidemiological and clinical studies focused on understanding HDP and its associated risk factors and clinical management, as well as long-term cardiovascular implications for the pregnant person, and impact on their offspring, including their elevated risk of hypertension and metabolic disorders in later life[3]. It has been reported that up to 50% of gHTN and cHTN cases show progression to the clinical symptoms of PE and siPE, respectively[4,5], with blood pressure returning to a pre-pregnancy baseline postpartum[1,6]. Risk factor analyses have shown that, although similar factors are present across the various subtypes of HDP, their relative strengths differ between subtypes[6]. This has fueled an ongoing debate regarding whether HDP represents a continuum of a single pathological process or comprises distinct entities, with no definitive consensus thus far.

Biomarker development studies provide some insight in this respect. There have been significant efforts in developing early PE detection markers, with the antiangiogenic marker s-Flt and PlGF (and the sFlt:PlGF ratio) effectively detecting early-onset (EO) cases within two weeks of occurrence[7]. This was a significant milestone for the establishment of early detection markers in PE; however, this marker is only useful after 20 weeks of gestation and is primarily effective for EO-PE near the time of onset, but not for late-onset (LO) PE or other HDP[8–10]. Thus, it has been suggested that HDP is a complex multifactorial syndrome, and, as a consequence, this biomarker fails to detect all cases. It is therefore unlikely that a single biomarker will capture the full spectrum of HDP subtypes, emphasizing the importance of understanding the pathophysiology of each[1,7,10–12]. To date, the majority of studies utilizing placental tissue RNA-seq have focused on subclassifying PE, without attention to other HDP subtypes. One recent study compared RNA-seq data from placental tissue of patients diagnosed with PE (n=16), siPE (n=7), cHTN (n=13), and non-hypertensive controls (n=12)[13]. Although this study did not contain gHTN samples, the authors identified a unique molecular signature for siPE, suggesting that this may represent a distinct disease entity compared to PE or cHTN[13].

Our group previously conducted in-depth characterization of primary PE at the clinical, pathological, cellular and molecular levels, and identified histopathological findings, cellular abnormalities, and molecular signatures which further subclassify this entity. Importantly, incorporating established criteria for the four main patterns of placental injury[14], based on the Amsterdam Workshop guidelines, enabled us to dissect PE further based on the placental pathology[15]. These four patterns of placental injury include: maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM), villitis of unknown etiology (VUE), and acute

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chorioamnionitis[14,16–18]. Among these patterns, MVM is most commonly associated with PE. MVM is thought to arise early in pregnancy, resulting from abnormal remodeling of maternal spiral arterioles by extravillous trophoblast, manifesting as decidual arteriopathy and leading to insufficient maternal blood flow to the placenta, and resulting compensatory changes in the chorionic villi, including accelerated villous maturation and infarction[14,17–19]. By leveraging these placental histopathological findings in our previous study, we found that, while MVM is found in PE presenting at any gestational age, EO- and LO-PE are differentially characterized by combinations of MVM with other distinct patterns of injury, namely with FVM in EO-PE and VUE in LO-PE[15]. This study clearly demonstrated the utility of placental pathology and molecular signatures in further understanding the etiology of these placenta-associated pregnancy disorders. Here, we apply a similar strategy, utilizing our more than 10-year-long pregnancy cohort and placental tissue RNA-sequencing data to compare the subtypes of HDP at the clinical, histological, and molecular levels to identify distinguishing characteristics of HDP subtypes.

Methods

Data and Code Availability

RNA-seq data has been deposited to the Gene Expression Omnibus database under accession numbers GSE186257, GSE234729, PRJNA1027377, and GSE303463. This paper does not report original code. The final code used for data processing and analysis has been deposited to github (https://github.com/mahorii/HDP-paper.git).

Ascertainment of clinical data and identification of the clinical study population: As part of an ongoing effort to examine the underlying causes of pregnancy complications, both low- and high-risk patients delivering at a UC San Diego-affiliated hospital are asked to consent to collection of clinical data and biospecimens (including placental tissue), for contribution to the obstetric registry and biobank at the Center for Perinatal Discovery. For all consented participants, specific demographic and clinical data are extracted from the medical record, including: maternal age, race and ethnicity, gravidity and parity, maternal height and weight (to calculate body mass index), gestational age at delivery, fetal sex, birthweight, mode of delivery, blood pressure measurements, and laboratory tests conducted during the pregnancy. Using this information, clinical diagnosis of hypertensive disorders of pregnancy and diabetes mellitus are adjudicated by two practicing obstetricians. For the purposes of the present analyses, sex-specific birthweight percentiles for gestational age were calculated using Fenton growth curves[20]. Small for gestational age was categorized as a birthweight < 10th percentile for gestational age.

The present study is a nested case-control study of participants with hypertensive disorders of pregnancy from our obstetric registry, who delivered between January 2010 – December 2023. Patients were included in the present study if their adjudicated hypertensive diagnosis included any of the following: (1) Chronic hypertension (cHTN), defined as documented hypertension (≥140/90 mm Hg) prior to 20 weeks of pregnancy with no signs or symptoms of preeclampsia during pregnancy (per ACOG definitions)[4]; (2) Gestational hypertension (gHTN), documented hypertension (≥140/90 mm Hg) after 20 weeks of pregnancy through

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delivery with no signs or symptoms of preeclampsia; (3) Superimposed preeclampsia (siPE), defined as chronic hypertension with evidence of proteinuria (≥300 mg), impaired liver function (twice the transaminase upper limit), low platelets ($\leq 1.0\times10^5$ cells/μl), or renal insufficiency (Cr≥1.2 mg/dL) after 20 weeks gestation; or (4) Preeclampsia (PE), new onset hypertension (≥140/90 mm Hg) after 20 weeks gestation, with evidence of proteinuria, impaired liver function, low platelets, or renal insufficiency after 20 weeks gestation. Both mild and severe hypertension and preeclampsia were included. Participants were excluded from analysis if they had a multiple gestation pregnancy or were missing birth outcome data, BMI prior to 20 weeks gestation, placental pathology, or adjudicated diabetes diagnosis. The final analytic samples included 266 cHTN, 259 gHTN, 281 siPE, and 497 PE patients.

Histopathological examination and classification: Placental examination, including gross and histologic examinations, were performed either through the hospital (for patients with a clinical indication for placental exam, e.g. diabetes, preeclampsia, or fetal growth restriction, etc.) or through the research core (for patients without an exam indication). Regardless of the route of examination, all placentas had histopathologic examination performed by one of two perinatal pathologists, based on the Amsterdam Consensus Statement[18]. Minimal obstetric information was available at the time of histologic evaluation, including gestational age, mode of delivery, and indication for placental examination, if applicable. The trimmed weight of the unfixed placental disc without the attached cord and membranes was measured and categorized as small (≤10th percentile for gestational age), normal (11–89th percentile), or large (≥90th percentile)[21].

For the present investigation, three primary lesions most often associated with hypertensive disorders of pregnancy were considered. Maternal vascular malperfusion (MVM) was defined as a small placenta with at least one of four vascular findings (accelerated villous maturation, infarction, decidual vasculopathy, or retroplacental/marginal hematoma), consistent with the traditionally used definition of MVM[15]. An additional definition of MVM that included any two vascular findings, regardless of placental size, was also considered. Any MVM captured either the traditional or secondary definition. The traditional definition was used for primary analyses, including RNA-seq analysis. Fetal vascular malperfusion (FVM) was defined as the presence of fetal vascular thrombosis or avascular villi, in the absence of villitis. Villitis of unknown etiology (VUE) was defined as focal, patchy or extensive villitis (infiltration of chorionic villi with chronic inflammatory cells), while severe VUE was defined as villitis associated with avascular villi (FVM) or perivillous fibrin. Pure VUE describes VUE without evidence of MVM (traditional definition). Normal pathology was therefore defined as any case not meeting the criteria for MVM (traditional definition), FVM, or VUE.

Clinical data analysis: In order to examine clinical and pathological differences between sub-types of HDP, clinically and biologically relevant pairwise comparisons were specified a priori: (1) examination of non-preeclamptic hypertension (cHTN vs. gHTN); (2) preexisting hypertension, with or without progression to preeclampsia (cHTN vs. siPE); (3) new onset hypertension, with or without progression to preeclampsia (gHTN vs. PE); and (4) preeclampsia sub-types (siPE vs. PE). These comparisons were chosen to elucidate

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differences between chronic and new onset hypertension, and hypertension and preeclampsia via clinically relevant pathways. Demographic, clinical, and pathological differences were compared between hypertensive disorder sub-groups using chi-square, pairwise t-test, or pairwise Wilcoxon-rank-sum, as appropriate. Adjustment for BMI and gestational age was considered in sub-analyses, as appropriate, using generalized linear or logistic models. Given that the directionality of the association between placental lesions and hypertensive outcomes are unknown, and that pathological findings can only be determined at the time of delivery, placental lesions were subsequently treated as the outcome and compared across hypertensive groups using log-binomial regression adjusting for maternal age, BMI, nulliparity, and diabetes, to estimate prevalence ratios with 95% confidence intervals. FVM and VUE models were additionally adjusted for gestational age at delivery. Models that did not converge were re-run using log-Poisson regression with robust standard errors, as noted. All analyses were conducted using SAS 9.4; a p-value of < 0.05 was considered significant.

RNA isolation from placental tissue and RNA sequencing: Placental villous tissue, half-way between the chorionic and basal plates avoiding calcification, infarct, and other abnormal macroscopic findings, was preserved in RNAlater (ThermoFisher) and banked in our biorepository as previously described[22]. Placental tissue from singleton pregnancies with diagnosis of cHTN (n=13), gHTN (n=11), siPE (n=54), PE (n=67), and normal, non-hypertensive control (n=129) was used for RNA-sequencing. Total RNA was isolated using mirVana RNA Isolation Kit (ThermoFisher). RNA concentration was measured using Qubit RNA BR assay kit (ThermoFisher). RNA integrity was checked using RNA 6000 Nano chip, read by a 2100 bioanalyzer (Agilent). RNA samples included in this dataset had RIN values ranging from 5.5 to 9.2, with a median of approximately 7.8, indicating high RNA integrity across the dataset. RNA-seq libraries were prepared using either the TruSeq Stranded Total RNA Sample preparation kit with Ribo-Zero Gold (Illumina) by IGM Genomics Center at UC San Diego, or the KAPA RNA HyperPrep Kit with RiboErase (Roche). TruSeq libraries were pooled and sequenced on NovaSeq 6000 and the HyperPrep libraries on NovaSeq X 25B Flow Cell (Illumina) with PE150 to an average depth of 35–40 million reads per sample through the IGM Genomics Center at UC San Diego. Quality control was performed using multiQC (v1.17, RRID:SCR_014982)[23]. Strand specificity analysis using RSeQC[24] confirmed that the RNA-seq libraries were stranded, with the majority of reads aligning to the antisense strand.

Adapter trimming and quality filtering were performed using BBDuk (BBMap suite, RRID:SCR_016965) with the following parameters: ktrim=r, k=16, mink=11, hdist=1, tpe, tbo, qtrim=rl, and trimq=20, using a custom adapter reference file. Reads were mapped to the human genome (GRCh38, GENCODE release 44 with ERCC spike-in sequences added, RRID:SCR_014966) using STAR (v.2.7.11, RRID:SCR_004463)[25] with the following parameters: --runMode alignReads, --outSAMtype BAM SortedByCoordinate, --outSAMunmapped Within, and --quantMode GeneCounts. Feature quantification was performed using featureCounts (subread v.2.0.1, RRID:SCR_012919)[26] with parameters -p --countReadPairs -t exon -T 13 -g gene_id. Only Ensembl genes with ≥10 counts in at least 25% of samples were retained for downstream analysis. Normalization and differential expression analysis were performed using the R (v. 4.4.1) package DESeq2 (v. 1.44.0,

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RRID:SCR_000154)[27]. BiomaRt (v. 2.60.1) was used to convert Ensembl gene IDs to HUGO gene names[28], and gene set enrichment analysis was done using the R (v. 4.4.1) package FGSEA (v. 1.30.0) and gene set variation analysis was done using GSVA (v. 1.4.4)[29]. Gene ontology analysis was done through Enrichr[30]. Statistical analysis for GSVA was done using SPSS (v. 29.0.2.0, RRID:SCR_002865).

Study approval: Clinical data as well as placental samples were collected under the protocol for our Perinatal Biorepository, approved by the Human Research Protections Program Committee of the University of California San Diego Institutional Review Board (IRB number: 181917X).

Results

Clinical characteristics and outcomes by hypertensive group

To better understand the clinical signatures of HDP, we characterized HDP subgroups in our cohort (266 cHTN, 259 gHTN, 281 siPE, 497 PE). Clinical characteristics by hypertensive sub-group as well as birth outcomes are presented in Table 1. Minor differences were noted by subgroup in regard to maternal age, BMI, and parity, as patients with cHTN and siPE were slightly older, had higher BMIs, and higher gravidity and parity than those with gHTN and PE, respectively. Patients with siPE were also more likely than cHTN and PE patients to have pregestational diabetes. As for the birth outcomes by hypertensive subgroup, gestational age (GA) at delivery, birthweight, and birthweight percentile were lower, while rates of preterm birth (GA < 37 weeks at delivery), small for gestational age, and cesarean section were higher, for preeclampsia groups (PE and siPE) compared to their hypertension-only counterparts (gHTN and cHTN). GA at delivery was lower, while preterm birth rate, birthweight percentile and rates of cesarean section were higher, in siPE compared to PE. Furthermore, GA at delivery was slightly lower and rates of preterm birth was slightly increased in cHTN compared to gHTN, though no other material differences in clinical outcomes were noted within the hypertension-only group (gHTN and cHTN).

Overall, the differences in clinical characteristics between PE and siPE, as well as gHTN and cHTN were minor, suggesting similarities in clinical signatures within preeclampsia (PE and siPE) and hypertension (gHTN and cHTN).

Placental pathology differentiates hypertensive subgroups

Our previous study[15], and others[14,31], have pointed to the importance of incorporating placental pathology to subclassify and understand the etiology of pregnancy complications. We leveraged the placental histopathological data from our database to identify differences in pathological lesions among HDP subgroups.

Notably, no differences in placental pathology were identified between cHTN and gHTN subgroups (Table 2). Both siPE and PE patients were less likely to have normal placental pathology (35.9% and 43.7%) than cHTN (58.3%, p<0.001) and gHTN (53.7%, p=0.009) subgroups, respectively, while siPE was slightly less likely to have normal pathology compared to PE (p=0.04). The presence of MVM was significantly increased in both preeclampsia groups (siPE, 56.4% and PE, 42.5%) compared to their hypertension-only

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counterparts (cHTN, 21.6% and gHTN, 19.7%, all p<0.001). This pattern was consistent regardless of the MVM definition used. VUE was slightly more common in gHTN compared to PE, though differences were attenuated following adjustment for GA at delivery.

Following adjustment for clinical factors, MVM remained significantly more common in preeclampsia (Figure 1 and Table S1): MVM was 2.67 times more likely to be seen in siPE compared to cHTN (95% CI 2.08–3.43) and 2.28 times more likely in PE compared to gHTN (95% CI 1.74–3.01). Similar patterns were noted for MVM sub-types. Severe VUE was less likely to be noted in cHTN (aPR=0.58, 95%CI 0.35–0.96) and PE (aPR=0.58, 95% CI 0.37–0.92) compared to gHTN. No other differences in placental lesions were observed within the hypertension-only subgroups or for FVM findings.

Since MVM likely originates in early gestation, secondary to abnormal spiral artery remodeling, our data suggest that the pathophysiology of preeclampsia (PE and siPE) and hypertension (gHTN and cHTN) is not on a continuum, but rather, diverges early in pregnancy (before 20 weeks GA).

Distinct molecular differences within preeclampsia (PE vs. siPE) and within hypertension (gHTN vs. cHTN) subtypes

Given that clinical and histopathological data analyses revealed similar disease characteristics between PE and siPE within the preeclampsia subgroup and between gHTN and cHTN within the hypertension-only subgroup, we next investigated whether these similarities extended to the molecular level, utilizing both our previously-obtained as well as newly-obtained placental RNA-seq data.

In order to directly assess the molecular differences between hypertensive subgroups in an unsupervised manner, as well as to account for the batch effects of sequencing data, we first conducted gene set variation analysis (GSVA) using the Hallmark gene sets from human MSigDB to identify activated pathways, which provides scores of each pathway within a single sample[29]. When comparing gene sets within hypertension (gHTN and cHTN), we identified a total of 9, out of 50, statistically enriched gene sets in gHTN compared to cHTN. Among these 9 gene sets, 5 were immune associated pathways (Figure 2A), consistent with previous findings suggesting gHTN is associated with increased immune activation[32]. In contrast, when comparing PE and siPE, there were 7 gene sets significantly enriched, 2 in PE and 5 in siPE. Interestingly, siPE showed enrichment in epithelial mesenchymal transition (EMT), angiogenesis, KRAS signaling, and DNA damage-associated gene sets, and PE showed enrichment in ER stress pathway and the heme metabolism gene set (Figure 2B). These data suggest that, despite similar clinical manifestations, PE and siPE (as well as gHTN and cHTN) exhibit distinct molecular profiles.

We next aimed to identify key genes involved in the activated pathways in HDP subtypes by leading edge gene analysis. To do this, we first conducted differential expression analysis (DEA) and identified a total of 452 differentially expressed genes (DEG) (144 upregulated, and 308 down-regulated genes) in cHTN vs. gHTN, and a total of 338 DEGs (137 upregulated, and 201 down-regulated genes) in PE vs. siPE (Figure 2C, Table S2).

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We then conducted gene set enrichment analysis (GSEA) using the Hallmark gene sets from human MSigDB to identify the signatures of the activated pathways in a supervised manner, and identify the leading edge genes. We identified a total of 25 gene sets (23 enriched in gHTN, and 2 enriched in cHTN) in gHTN vs. cHTN comparisons (Figure S1A), and a total of 31 gene sets (23 enriched in PE and 8 enriched in siPE) in PE vs. siPE comparisons (Figure S1B). Interestingly, we identified stanniocalcin-1 (STC1) in the hypoxia gene set to be significantly reduced in cHTN, both in cHTN vs. gHTN (Log_2 fold-change =−1.31) and siPE vs. cHTN (Log_2 fold-change =0.99) comparisons, pointing to a consistent reduction of STC1 in cHTN placenta. Related to this finding, we also found cHTN placentas had increased expression of glutathione S-transferase alpha 3 (GSTA3, cHTN vs gHTN: Log_2 fold-change =1.03), which plays a role in detoxifying reactive oxygen species and protecting cells from oxidative damage[33]. However, STC1 and GSTA3 was not statistically different in PE vs. siPE comparisons. Similar results connecting the cHTN subgroup and its response to hypoxia was noted when comparing cHTN to control placentas, those without any associated hypertension. Of the four HDP subtypes, only cHTN did not show statistically significant enrichment in the hypoxia pathway induction compared to our normal control group (Figure S1C). These data suggest that the placentas in the cHTN subgroup may not experience the same low oxygen tension as the other HDP subgroups or may be responding to the hypoxic environment in a different way.

Lastly, we hypothesized that if there were similarities within hypertension-only (gHTN and cHTN) or the preeclampsia (PE and siPE) subgroups, we would see similar gene expression changes and activated pathways in the respective comparisons (gHTN vs. PE, and cHTN vs. siPE). Thus, we conducted DEA of gHTN vs. PE and cHTN vs. siPE and compared the up-regulated genes of PE and siPE, as well as the up-regulated genes of gHTN and cHTN. There were a total of 76 DEGs (7 upregulated, and 69 down-regulated genes) in PE vs. gHTN, and a total of 150 DEGs (46 upregulated, and 104 down-regulated genes) in siPE vs. cHTN, but no overlapping DEGs between these conditions (Figure 2D, Table S3). We also conducted GSEA on these gene lists using the Hallmark gene sets from human MSigDB to identify the activated pathways in preeclampsia development. Again, we hypothesized that if there are similarities within hypertension (gHTN and cHTN) or within preeclampsia (PE and siPE), we would see similar pathways activated in our respective comparisons (gHTN vs. PE, and cHTN vs. siPE). Again, we found no overlapping gene sets significantly enriched between gHTN vs. PE and cHTN vs. siPE (Figure S1D).

Overall, these data suggest that there are significant molecular differences in hypertension (gHTN and cHTN) as well as in preeclampsia (PE and siPE).

Molecular profiling suggests that gHTN and cHTN do not transition to PE or siPE

As stated previously, it has been reported that up to 50% of cases that manifest as hypertension (gHTN and cHTN) progress to preeclampsia (PE and siPE)[4,5]. However, our clinical data suggest that the progression to preeclampsia may not simply be a continuum of disease severity, but rather a distinct pathological onset. This hypothesis is supported by the finding that MVM, a pathological change that begins early in gestation, is significantly elevated in preeclampsia (PE and siPE) compared to hypertension (gHTN and cHTN).

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Thus, the pathways that lead to preeclampsia (PE and siPE) may differ from those that drive hypertension (gHTN and cHTN). To test this hypothesis, we analyzed placental transcriptome profiles from two perspectives. We reasoned that if disease progression follows a continuum, we would expect to see progressively larger gene expression changes from non-hypertensive controls to hypertensive cases (gHTN and cHTN), and then to preeclamptic cases (PE and siPE). Alternatively, if the disease subtypes arise from distinct etiologies, their gene expression patterns and activated pathways should differ.

Thus, we first conducted DEA, controlling for gestational age, BMI, fetal sex, and the sequencing batch, comparing preeclampsia (PE or siPE) or hypertension (gHTN or cHTN) against our non-hypertensive controls. Common DEGs between the two comparisons were filtered out. We found larger gene expression differences between the non-hypertensive control group and the hypertension (gHTN and cHTN) group compared to that of preeclampsia (PE or siPE) (Figure 3A, Table S4). We also conducted DEA between our non-hypertensive controls and hypertension (gHTN and cHTN) group, and between our hypertension (gHTN and cHTN) group and our preeclampsia (PE or siPE) group; however, there were no common DEGs within the respective comparisons.

We next compared gene set enrichment differences between preeclampsia (PE or siPE) or hypertension (gHTN or cHTN) and non-hypertensive control, using GSVA scores (Figure 3B–3C and Figure S2A–S2B). There was a total of 22 gene sets significantly enriched in the comparisons between non-hypertensive control, gHTN, and PE. Within those activated pathways, there were no gene sets showing a continuous increase in the GSVA scores from non-hypertensive control to gHTN, and then to PE. Instead, gHTN showed immune associated gene sets to be significantly highly activated. Likewise, there were a total of 19 gene sets significantly enriched in the comparisons between non-hypertensive control, cHTN, and siPE. Again, there were no gene sets showing a continuous increase in the GSVA scores from non-hypertensive control to cHTN, and then to siPE.

Together, these data suggest that hypertension-only disease (gHTN and cHTN) does not show strong transcriptomic evidence of continuously progressing to preeclampsia (PE and siPE).

Placental tissue RNA-seq reveals molecular signature of MVM to be similar in PE and siPE

Since MVM lesions were predominantly found in placenta from preeclampsia (PE and siPE), we next questioned whether there were any transcriptomic differences between placenta with and without MVM in PE or siPE subgroups that may point to the specific disease pathway, using the same approach as the previous section.

We first assessed differences in an unsupervised manner using GSVA scores to identify enriched pathways. Strikingly, within MVM placentas (PE: n=40, siPE: n=22), GSVA did not show any differences in PE vs. siPE; however, within non-MVM placentas (PE: n= 27, siPE: n=32), we noted significant enrichment in EMT, heme metabolism, KRAS signaling, PI3 kinase signaling via AKT to mTORC1, TGFβ signaling, hypoxia, and unfolded protein response pathways in PE vs. siPE (Figure 4A). Additionally, DEA was again done by controlling for gestational age, BMI, fetal sex, and the sequencing batch, and we did not

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see any DEGs when comparing siPE and PE groups within MVM (Figure 4B, Table S5), suggesting that PE and siPE are similar in molecular signature when MVM is noted in the placenta. In contrast, we found 2,051 DEGs when comparing siPE and PE within non-MVM placentas (n=791 highly expressed in siPE, and n=1,260 highly expressed in PE) (Figure 4B, Table S5). We again conducted GSEA to identify significant leading-edge genes. Compared to non-MVM PE, we found non-MVM siPE placenta showed significantly enriched gene expression in extracellular matrix organization associated genes in the EMT pathway, while non-MVM PE placenta were significantly enriched in genes BNIP3L, Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2 (CITED2), phosphoglycerate kinase 1 (PGK1), AK4, NDRG1, and vascular endothelial growth factor A (VEGFA), which are associated with cellular response to hypoxia (Table S6)[34–42].

Finally, we assessed differences between MVM and non-MVM within the preeclampsia subtypes. We found that EMT, KRAS signaling, and reactive oxygen species were significantly different between MVM and non-MVM PE placentas (Figure 4C); however, no differences were noted in siPE by GSVA. We next conducted DEA and found that there were 282 (153 up-regulated and 129 down-regulated) and 154 (131 up-regulated and 23 down-regulated) DEGs in PE and siPE, respectively (Figure 4D, Table S7). GSEA identified notable differences in pathways with several interesting leading-edge genes. Specifically, leading edge genes in heme metabolism were significantly enriched with autophagy associated genes, F-Box Protein 7 (FBXO7), BCL2 interacting protein 3 like (BNIP3L), forkhead box O3 (FOXO3), and optineurin (OPTN), in placentas from non-MVM PE when compared to MVM PE (Table S8). On the other hand, MVM placentas from siPE patients were significantly enriched in hypoxia gene set, including adenylate kinase 4 (AK4) and N-myc downstream-regulated gene 1 (NDRG1) (Table S9), genes that are known to be upregulated in response to low oxygen tension[34–36]. These findings suggest that placenta with and without MVM show distinct molecular signatures in both PE and siPE, with larger changes observed in PE than siPE.

Overall, these findings indicate that, although the presence or absence of MVM seems to have a somewhat larger influence on PE than siPE, MVM demonstrates a common molecular signature regardless of the preeclampsia subtype, whereas in the absence of MVM, distinct molecular signatures are seen. Thus, placental histopathological findings, particularly the presence of MVM, could better inform the molecular pathophysiology of the HDP subtypes.

Discussion

We previously reported that incorporating placental histopathological findings is important for dissecting primary PE, a multifactorial pregnancy syndrome[15]. Thus, in the present study, we applied a similar evaluation to a broader group of HDP patients to enhance our current understanding of this disease. Using our more than ten-year cohort of over 1300 patients with HDP, we analyzed maternal clinical features and found only minor differences between HDP subtypes, including maternal age, BMI, and parity. These findings may reflect older maternal age and higher BMI influencing preexisting hypertensive conditions. The only significant difference we noted was higher prevalence of pregestational diabetes

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in siPE compared to cHTN and PE patients. Clinically meaningful observations were made regarding birth outcomes, which were overall significantly worse in preeclampsia (PE and siPE) than in hypertension (gHTN and cHTN), as expected. Thus, we conclude that, within preeclampsia (PE and siPE) and hypertension-only (gHTN and cHTN) groups, clinical characteristics and birth outcomes are similar, but that birth outcomes are worse in preeclampsia (PE and siPE) compared to hypertension-only (gHTN and cHTN) group.

Subsequently, we investigated the pathological characteristics of placental injury in the four HDP subtypes, characteristics that have been evaluated less commonly within these distinct subtypes. We found that MVM was significantly increased in preeclampsia (PE and siPE), compared to their respective hypertension (gHTN and cHTN) subtypes. Further, normal placental pathology was more common in hypertension-only (gHTN and cHTN) cases which did not ultimately progress to preeclampsia (PE and siPE). This finding reflects previous reports suggesting that the worst birth outcomes, such as preterm birth and small for gestational age, are associated with a high prevalence of MVM[19,31].

We next conducted placental RNA-seq to test whether the absence of significant clinical differences within preeclampsia (PE and siPE) and within hypertension (gHTN and cHTN) correlated to similar molecular signatures in the placenta. Interestingly, when comparing gHTN vs. cHTN or PE vs. siPE, we noted different pathways activated, different DEGs between the subtypes, and no overlapping DEGs or pathways between the PE and HTN groups, suggesting that the four HDP subtypes have unique molecular signatures. One interesting finding was the lack of a gene expression response to hypoxia in cHTN-associated placentas. As noted earlier, we found reduced expression of STC1, a downstream gene turned on in response to the activation of the hypoxia inducible factor (HIF) pathway, in cHTN. STC1 is known to be upregulated in BeWo cells cultured under hypoxia through the activation of HIF[43]. We also noted GSTA3, a gene involved in detoxifying reactive oxygen species and protecting cells from oxidative damage[33], to be significantly upregulated in cHTN. The gene expression profiles of these hypoxia related genes, along with our finding that the GSVA hypoxia scores of cHTN are similar to non-hypertensive controls, suggest cHTN placentas may not be experiencing hypoxia or respond to hypoxia in a distinct manner compared to gHTN.

We then asked whether we could see the molecular footprint of the progression of hypertension (gHTN and cHTN) to preeclampsia (PE and siPE), or whether these HDP subtypes have distinct origins. We first tested the assumption that disease progression would likewise show a mirrored gene expression continuum. We contrasted our non-hypertensive controls to both our hypertension-only subtypes (gHTN or cHTN), as well as to our preeclampsia subtypes (PE and siPE). Instead of a gene expression continuum, we noted that, compared to our non-hypertensive controls, both our hypertension-only subtypes (gHTN or cHTN) had larger gene expression changes than either of the PE subtypes (PE or siPE). Additionally, we used GSVA scores to investigate the activated pathways in an unsupervised manner. If HDP disease subtypes existed on a continuum, we would expect a linear increase in GSVA scores compared to the non-hypertensive state. However, there were no gene sets showing a linear increase in GSVA scores, suggesting no pathways were being activated in a linear fashion. Considering the fact that MVM is thought to start at an early

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stage of pregnancy, secondary to abnormal vascular remodeling[14,17,18], and that MVM is significantly more highly enriched in placentas with preeclampsia (PE and siPE) compared to hypertension (gHTN and cHTN), as well as the transcriptomic data presented here, we propose that preeclampsia (PE and siPE) and hypertension (gHTN and cHTN) have distinct pathophysiologic origins.

Finally, since we noted MVM as the key pathological feature associated with preeclampsia (PE and siPE), in order to identify key pathways which distinguish each condition, we set out to compare the transcriptional changes between these placentas, comparing those with or without MVM. Strikingly, we found similarities in the transcriptomes of PE and siPE placentas with MVM; however, significant differences were noted in non-MVM placentas between PE and siPE. These data are intriguing, and suggest that PE and siPE have a common molecular signature in the presence of MVM, but diverge in the absence of MVM. Our current data, as well as previous report[13], noted distinct molecular signatures of PE and siPE when the placental histopathological findings are not considered, suggesting the importance of incorporating placental histopathological findings to further elucidate the molecular pathophysiology of the disease.

Together, our clinical and placental transcriptomic data suggest that the factors leading to preeclampsia may not be on a continuum with hypertension, but rather separate in origin at disease onset. Considering the key feature of MVM origin as abnormal spiral artery remodeling, which is mediated by extravillous trophoblast, and given that extravillous trophoblast play a crucial role in early placentation, we believe that incorporation of placental examination and identification of distinct patterns of placental injury are key to further delineate HDP disease etiology.

While we believe our findings are meaningful, several limitations should be acknowledged. Our sample collection was selective as recruitment was limited to patients delivering at our university affiliated hospital, which is a tertiary care center that treats patients with high-risk pregnancies. In addition, within the high-risk patients, clinical coordinators prioritize the recruitment of patients with higher risk for adverse effects, as reflected by the older maternal age and higher C-section rate in our study participants. Although this recruitment strategy may introduce biases and limit generalizability to broader study populations, it also provides the opportunity to capture and study a broad range of HDP subtypes in populations at higher risk for developing these conditions. Our cohort did not contain a large number of RNA-seq samples from cHTN and gHTN with MVM to enable a comparison of hypertension placentas with and without MVM. We are continuing to collect placental tissue samples from patients with hypertension and project that our follow-up studies will be sufficiently powered to compare the differences among the MVM-containing HDP subtypes. We concede that our hypothesis of a linear progression from hypertension to preeclampsia is influenced by the availability of placental tissue representing only the terminal stage of the disease. We acknowledge that the best way to test disease progression would be to acquire real time data throughout pregnancy. However, currently, the only feasible method to evaluate progression of disease during pregnancy is through maternal blood profiling. We believe future studies involving not only assessment of placental tissue, but also utilizing

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longitudinal maternal blood samples as well as placental imaging throughout pregnancy are necessary to better evaluate disease progression and elucidate disease etiology.

To date, in-depth studies of the etiology of HDP have been limited to PE, and have generally not included other HDP subtypes. While existing data suggest that abnormal trophoblast differentiation contributes to disease onset, these studies lack in-depth mechanistic insight into the specific cell types and pathways driving its development. Because the clinical characteristics are similar between PE and siPE, as well as between gHTN and cHTN, it is challenging to distinguish between these HDP etiologies without pathological or molecular data. Thus, to fully understand the disease, it is necessary to evaluate not only the clinical phenotype, but also probe the placenta at the tissue, cellular, and molecular levels. We believe our study confirms previous findings, but also contributes novel findings, highlighting the need for utilizing placental pathology to understand the etiology of specific HDP subtypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and acronyms

HDP	Hypertensive disorder of pregnancy
cHTN	Chronic hypertension
gHTN	Gestational hypertension
PE	Preeclampsia
siPE	Superimposed preeclampsia
MVM	Maternal vascular malperfusion
FVM	Fetal vascular malperfusion
VUE	Villitis of unknown etiology
GA	Gestational age

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EO-PE	Early onset preeclampsia
LO-PE	Late onset preeclampsia
BMI	Body mass index
DEG	Differentially expressed genes
DEA	Differential expression analysis
GSVA	Gene set variation analysis
GSEA	Gene set enrichment analysis
EMT	Epithelial mesenchymal transition

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What are the clinical implications:

Our study has several important clinical implications in understanding hypertensive disorders of pregnancy (HDP). Our placental histopathology analysis found maternal vascular malperfusion (MVM), which originates in early gestation, is significantly elevated in preeclampsia (PE and siPE) compared to hypertension (gHTN and cHTN). Transcriptome data showed that preeclampsia (PE and siPE) is a distinct condition rather than a severe form of hypertension (gHTN and cHTN). Thus, our data suggest that preeclampsia and hypertension-only subtypes likely have distinct pathophysiologic origins rather than existing on a disease continuum. These findings indicate that incorporation of placental histopathological findings can help elucidate underlying disease etiology, refine diagnosis, and develop targeted treatment approaches in the future. This work provides a foundation for future HDP management, including the development of early diagnostic biomarkers to enable subtype specific prevention and treatment strategies.

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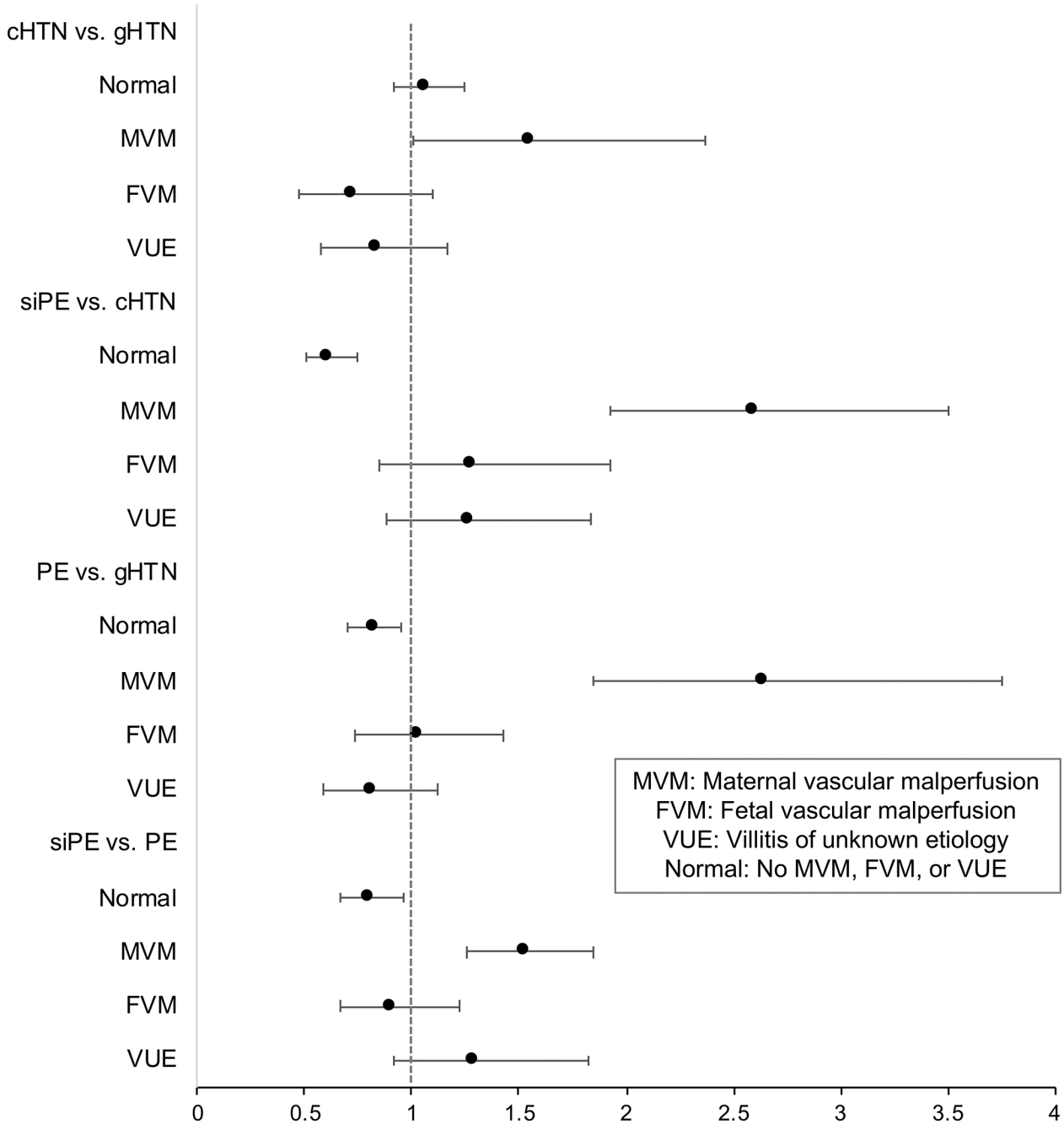

Figure 1:

Comparison of specific placental lesions by hypertensive disorders of pregnancy diagnosis: prevalence ratios and 95% confidence intervals from log-binomial regression models adjusted for maternal age, BMI, nulliparity, and diabetes; VUE additionally adjusted for GA.

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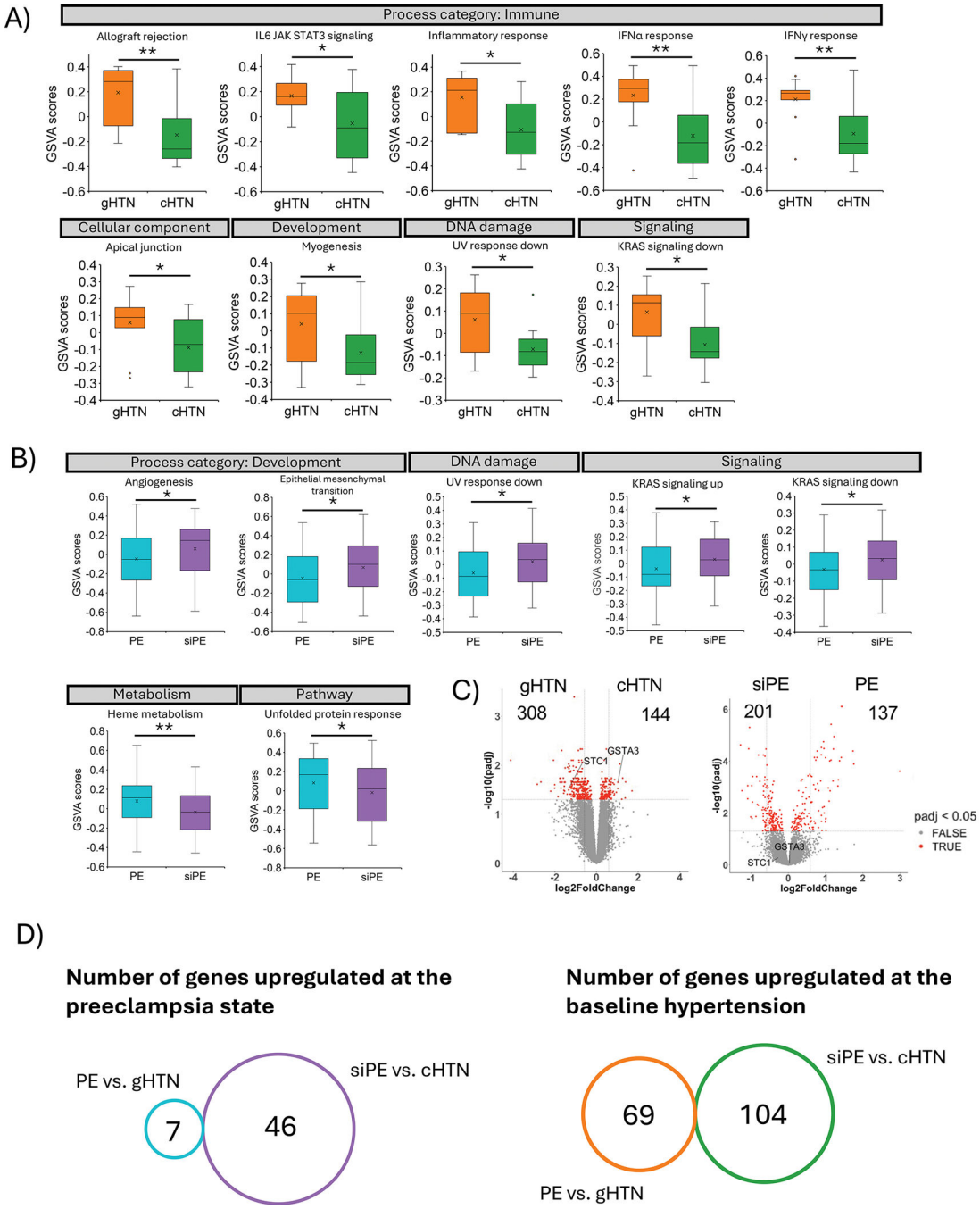

Figure 2:

Box plots showing the significantly different GSVA MSigDB hallmark gene sets between A) gHTN vs. cHTN, B) PE vs. siPE. Line within box represents median, "X" mark indicates the mean, top and bottom of box indicate interquartile rage with the bottom and top edge marking the first and third quartiles respectively. gHTN vs. cHTN: Mann-Whitney U test was used for allograft rejection, inflammatory response, IFNα response, and IFNγ response; t-test was used for IL6 JAK STAT3 signaling, apical junction, myogenesis, UV response down, and KRAS signaling down based on the data distribution. PE vs. siPE: Mann-Whitney

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U test was used for angiogenesis, epithelial mesenchymal transition, UV response down, heme metabolism, and unfolded protein response; t-test was used for KRAS signaling down and KRAS signaling up, based on the data distribution. C) Volcano plot displaying both up-regulated and down-regulated differentially expressed genes (DEGs) from comparison of cHTN vs. gHTN (left panel) and siPE vs. PE (right panel). Adjusted p-value<0.05 are highlighted in red. D) Venn diagram shows that there are no overlapping DEG in up-regulated or down-regulated genes in these comparisons. *p<0.05, **p<0.01

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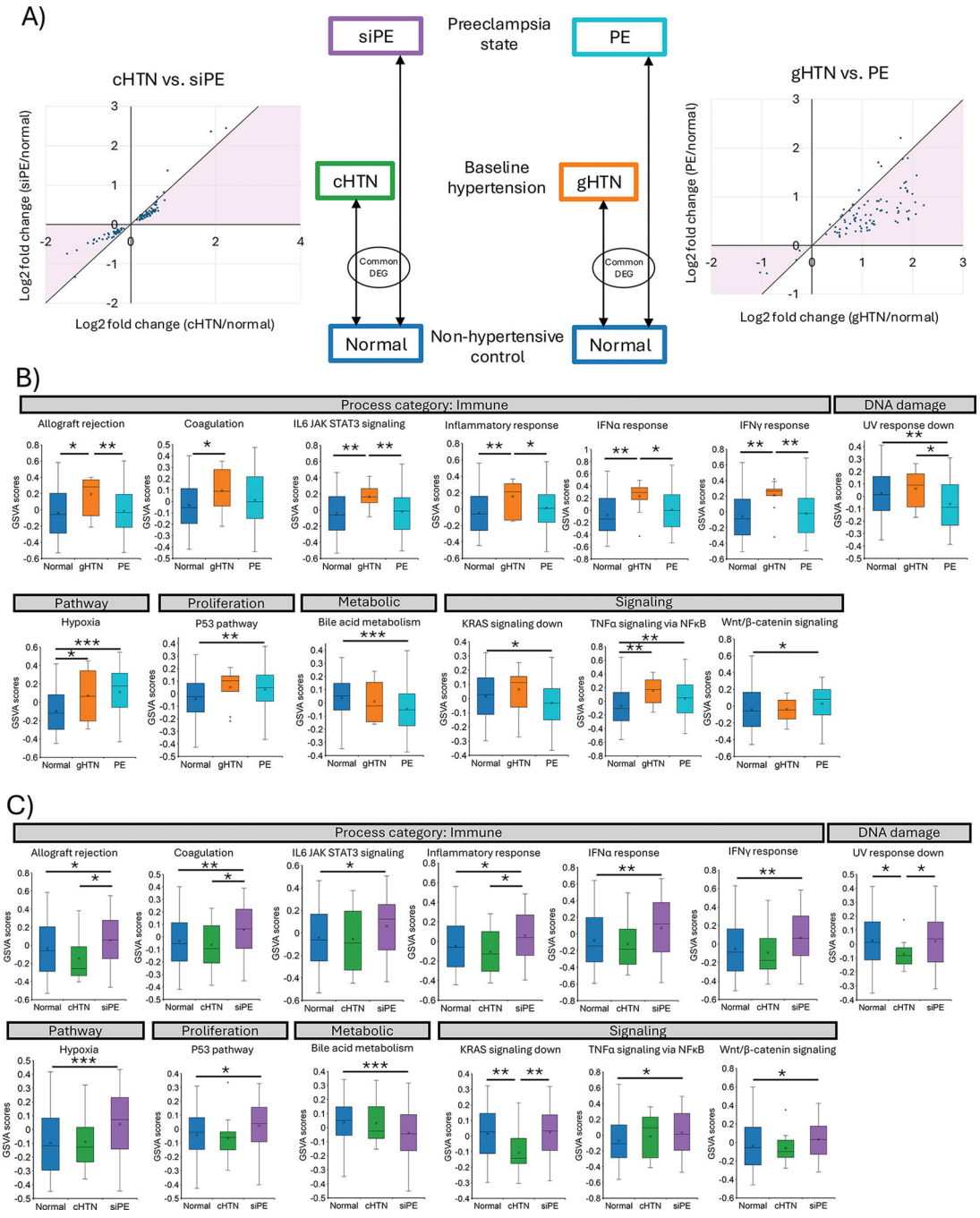

Figure 3:

A) Scatter plot displaying the Log2 fold change of the common differentially expressed genes contrasted from non-hypertensive controls to hypertension (gHTN or cHTN) in x axis, or to preeclampsia (PE or siPE) in y-axis. Pink shaded area indicates higher gene expression in hypertension subgroup compared to preeclampsia subgroup. B) Box plots showing the significantly different GSVA MSigDB hallmark gene sets between normal (non-hypertensive control), gHTN, and PE, and C) between normal (non-hypertensive control), cHTN, and siPE. Line within box represents median, “X” mark indicates the mean,

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top and bottom of box indicate interquartile rage with the bottom and top edge marking the first and third quartiles respectively. Pathways shown here are common pathways between B) and C). Other pathways are listed in Supplementary Figure 2. Normal vs. gHTN: Mann-Whitney U test was used for allograft rejection, coagulation, IL6 JAK STAT3 signaling, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, bile acid metabolism, KRAS signaling down, TNFA signaling via NFκB, and Wnt/β-catenin signaling; t-test was used for p53 pathway, based on the data distribution. Normal vs. PE: Mann-Whitney U test was used for allograft rejection, coagulation, IL6 JAK STAT3 signaling, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, bile acid metabolism, KRAS signaling down, TNFα signaling via NFκB, and Wnt/β-catenin signaling; t-test was used for p53 pathway, and UV response up, based on the data distribution. gHTN vs PE: Mann-Whitney U test was used for allograft rejection, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, and Wnt/β-catenin signaling; t-test was used for coagulation, IL6 JAK STAT3 signaling, p53 pathway, bile acid metabolism, KRAS signaling down, and TNFA signaling via NFκB, based on the data distribution. Normal vs. cHTN: Mann-Whitney U test was used for allograft rejection, coagulation, IL6 JAK STAT3 signaling, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, bile acid metabolism, KRAS signaling down, TNFα signaling via NFκB, and Wnt/β-catenin signaling; t-test was used for p53 pathway, based on the data distribution. Normal vs. siPE: Mann-Whitney U test was used for allograft rejection, coagulation, IL6 JAK STAT3 signaling, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, bile acid metabolism, KRAS signaling down, TNFα signaling via NFκB, and Wnt/β-catenin signaling; t-test was used for up p53 pathway, based on the data distribution. cHTN vs siPE: Mann-Whitney U test was used for allograft rejection, IL6 JAK STAT3 signaling; t-test was used for coagulation, inflammatory response, IFNα response, IFNγ response, UV response down, hypoxia, p53 pathway, bile acid metabolism, KRAS signaling down, TNFα signaling via NFκB, and Wnt/β-catenin signaling, based on the data distribution. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

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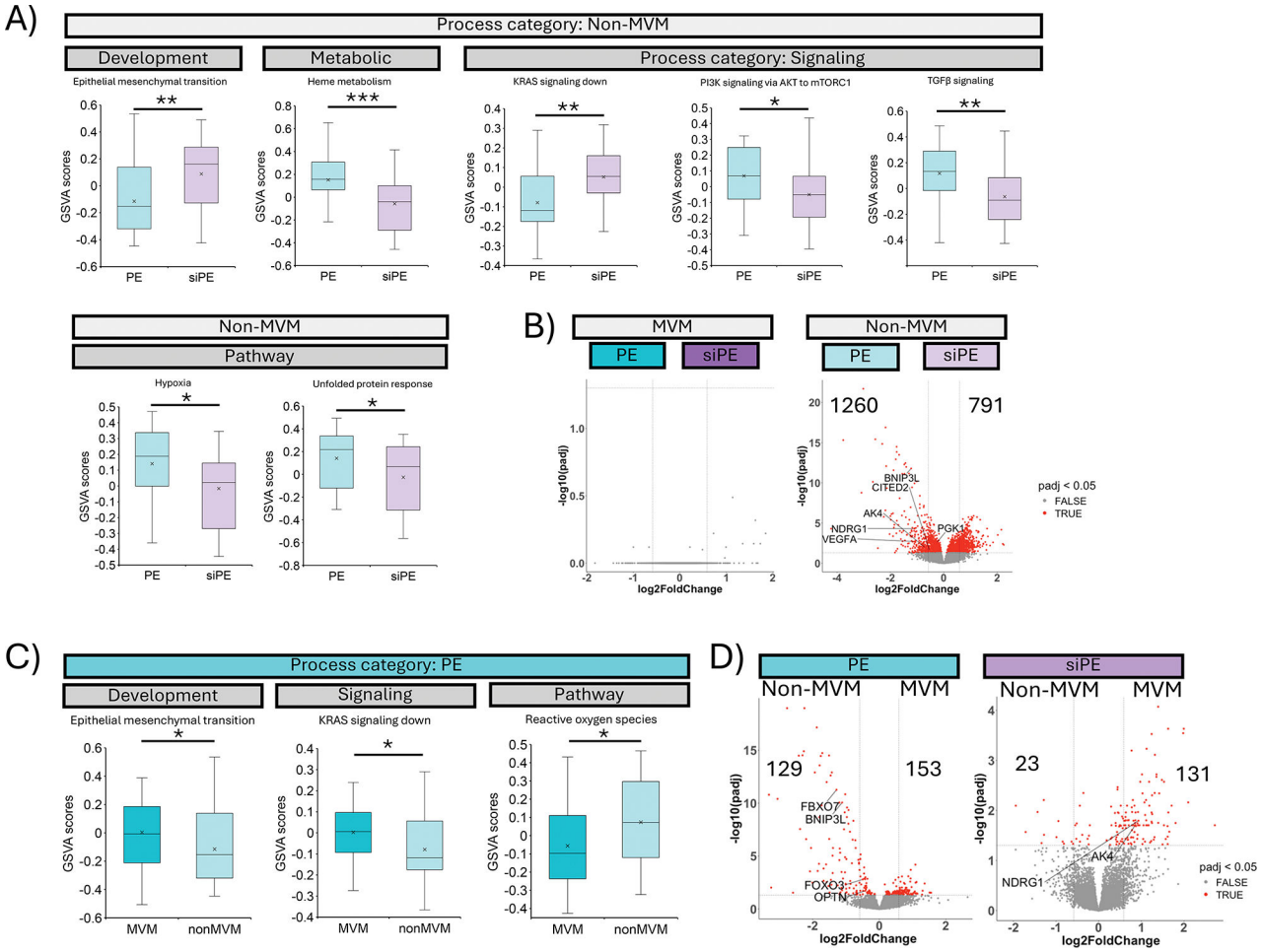

Figure 4:

A) Box plots showing the significantly different GSVA MSigDB hallmark gene sets between PE-nonMVM and siPE-nonMVM. There were no significant gene sets between PE-MVM and siPE-MVM. Line within box represents median, “X” mark indicates the mean, top and bottom of box indicate interquartile rage with the bottom and top edge marking the first and third quartiles respectively. Mann-Whitney U test was used for EMT, unfolded protein response; t-test was used for heme metabolism, KRAS signaling down, PI3K signaling via AKT to mTORC1, TGFβ signaling, and hypoxia, based on the data distribution. B) Volcano plot displaying both up-regulated and down-regulated DEGs from comparison of PE vs. siPE of MVM (left panel) and non-MVM (right panel). Adjusted p-value<0.05 are highlighted in red. Dotted lines indicates adjusted p-value at 0.05 and fold change at 1.5. C) Box plots showing the significantly different GSVA MSigDB hallmark gene sets between PE-MVM and PE-nonMVM. There were no significant gene sets between siPE-MVM and siPE-nonMVM. Line within box represents median, “X” mark indicates the mean, top and bottom of box indicate interquartile rage with the bottom and top edge marking the first and third quartiles respectively. Mann-Whitney U test was used for EMT; t-test was used for KRAS signaling down and reactive oxygen species, based on the data distribution. D) Volcano plot displaying both up-regulated and down-regulated DEGs from comparison of MVM vs. non-MVM of PE (left panel) and siPE (right panel). Adjusted p-value<0.05 are

highlighted in red. Dotted lines indicates adjusted p-value at 0.05 and fold change at 1.5. *p<0.05, **p<0.01, ***p<0.001.

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Table 1:

Clinical characteristics and birth outcomes by hypertensive outcome group

Characteristic	Hypertensive Outcome Group				Pairwise Comparisons of Interest (p-value)[1]			
	cHTN 266 (20.4%)	gHTN 259 (19.9%)	siPE 281 (21.6%)	PE 497 (38.1%)	cHTN v. gHTN	cHTN v. siPE	gHTN v. PE	siPE v. PE
Maternal Demographics								
Age (mean, SD)	32.9 (5.1)	31.8 (5.7)	33.2 (6.1)	31.5 (6.2)	**0.04**	0.52	0.52	< **0.001**
Race/ethnicity (n, %)					0.33	0.19	0.21	0.29
NH White	95 (37.5)	110 (45.4)	82 (30.5)	178 (36.9)				
NH Black	24 (9.5)	14 (5.8)	19 (7.1)	26 (5.4)				
Hispanic	98 (38.7)	85 (35.1)	132 (49.1)	206 (42.7)				
Asian/PI	30 (11.9)	27 (11.2)	29 (10.8)	55 (11.4)				
Mixed	6 (2.4)	6 (2.5)	7 (2.6)	17 (3.5)				
BMI (mean, SD)	34.7 (10.8)	30.3 (8.2)	34.3 (8.9)	29.4 (7.9)	< **0.001**	0.60	0.21	< **0.001**
Pregnancy Characteristics								
Gravidity (median, IQR)	2 (1, 4)	2 (1, 3)	2 (1, 4)	2 (1, 3)	**0.003**	0.57	0.77	**0.003**
Parity (median, IQR)	1 (0, 2)	1 (0, 1)	1 (0, 2)	0 (0, 1)	**0.008**	0.47	0.33	**0.003**
Nulliparous (n, %)	100 (37.6)	126 (48.6)	126 (45.0)	280 (56.3)	**0.01**	0.08	**0.04**	**0.002**
Fetal sex, male (n, %)	141 (53.0)	137 (52.9)	155 (55.2)	242 (48.7)	0.98	0.61	0.27	0.08
Diabetes (n, %)					0.69	**0.008**	0.84	< **0.001**
None	183 (68.8)	187 (72.2)	166 (59.1)	349 (70.2)				
Pre-gestational (T1 or T2)	54 (20.3)	46 (17.8)	90 (32.0)	96 (19.3)				
Gestational diabetes mellitus	29 (10.9)	26 (10.0)	25 (8.9)	52 (10.5)				
Birth Outcomes								
Gestational age (mean, SD)	38.0 (2.2)	38.8 (1.8)	35.3 (3.8)	36.7 (3.3)	**0.003**	< **0.001**	< **0.001**	< **0.001**

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Characteristic	Hypertensive Outcome Group				Pairwise Comparisons of Interest (p-value)[1]			
	cHTN 266 (20.4%)	gHTN 259 (19.9%)	siPE 281 (21.6%)	PE 497 (38.1%)	cHTN v. gHTN	cHTN v. siPE	gHTN v. PE	siPE v. PE
Preterm birth, < 37wks. (n, %)	50 (18.8)	26 (10.0)	158 (56.2)	192 (38.6)	**0.004**	**< 0.001**	**< 0.001**	**< 0.001**
Birthweight, g (mean, SD)	3257 (650)	3389 (530)	2619 (996)	2747 (913)	0.07	**< 0.001**	**< 0.001**	**0.04**
Birthweight percentile (mean, SD)	63.9 (24.1)	61.2 (25.9)	54.0 (32.7)	46.7 (32.0)	0.29	**< 0.001**	**< 0.001**	**< 0.001**
Small for gestation age (n, %)	4 (1.5)	5 (1.9)	36 (12.9)	88 (17.7)	0.75	**< 0.001**	**< 0.001**	0.08
Mode of delivery[2]					0.06	**< 0.001**	**< 0.001**	**0.002**
C-Section	133 (50.0)	108 (41.7)	185 (66.1)	272 (54.7)				
Vaginal	133 (50.0)	151 (58.3)	95 (33.9)	225 (45.3)				

[1] p-value based on chi-square, t-test (continuous variables reported as mean, SD), or Wilcoxon-rank-sum (variables reported as median, IQR), as appropriate

[2] 1 participant (siPE) needed a late D&E

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Table 2:

Placental pathology by hypertensive outcome group

Pathology lesion n (%)	Hypertensive Outcome Group				Pairwise Comparisons of Interest (p-value)			
	cHTN 266 (20.4%)	gHTN 259 (19.9%)	siPE 281 (21.6%)	PE 497 (38.1%)	cHTN v. gHTN	cHTN v. siPE	gHTN v. PE	siPE v. PE
Normal[a]	155 (58.3)	139 (53.7)	101 (35.9)	217 (43.7)	0.29	**< 0.001**	**0.009**	**0.04**
Any MVM (definition 1 or 2 below)	57 (21.6)	48 (19.7)	158 (56.4)	211 (42.5)	0.41	**< 0.001**	**< 0.001**	**< 0.001**
MVM1 (small placenta + vascular)	45 (17.1)	31 (12.1)	118 (42.3)	159 (32.2)	0.11	**< 0.001**	**< 0.001**	**0.005**
MVM2: ≥2 vascular findings	25 (9.4)	30 (11.6)	109 (38.8)	141 (28.4)	0.41	**< 0.001**	**< 0.001**	**0.003**
FVM (no VUE)	33 (12.4)	43 (16.7)	56 (19.9)	104 (20.9)	0.17	**0.02** [c]	0.16	0.74
Pure FVM (no MVM)	26 (9.8)	39 (15.1)	24 (8.5)	65 (13.1)	0.06	0.62	0.45	0.05
VUE (any)	49 (18.4)	57 (22.1)	50 (17.8)	73 (14.7)	0.30	0.85	**0.01** [d]	0.25
Pure VUE (no MVM)	38 (14.3)	47 (18.3)	36 (12.8)	53 (10.7)	0.22	0.60	**0.004** [e]	0.37
Severe VUE[b]	23 (8.7)	36 (13.9)	24 (8.5)	36 (7.2)	0.05	0.96	**0.003** [f]	0.51

[a] No MVM (1), FVM, or VUE

[b] Villitis associated with FVM or perivillous fibrin deposition

[c] p = 0.26 controlling for GA at delivery; GA at delivery p = 0.004

[d] p = 0.12 controlling for GA at delivery; GA at delivery p = 0.02

[e] p = 0.10 controlling for GA at delivery; GA at delivery p = 0.003

[f] p = 0.01 controlling for GA at delivery; GA at delivery not significant (p = 0.45)